REMARKS

I. Status of the Claims and Formal Matters

Claims 1-7, 9, 10, 12-35, and 37-39 are pending in the application. Applicants have amended claims 1 and 9, cancelled claims 2 and 6 and added claims 96-98. Accordingly, following entry of this paper, claims 1, 3-5, 7, 9, 10, 12-35, 37-39, and 96-98 will be pending in this application.

Support for the amendments and new claims presented herein can be found throughout the application as originally filed and from the pending and original claims. Exemplary support for the amendments is as follows. Support for new claim 96 can be found, e.g., at paragraph 29 of U.S. Patent Publication No. 2007/0178454, which discloses the use of polysome systems in the alternative. Support for new claim 97 can be found, e.g., at paragraph 73 of U.S. Patent Publication No. 2007/0178454. Support for new claim 98 can be found, e.g., at paragraph 73 and Example 8 of U.S. Patent Publication No. 2007/0178454, and in U.S. Patent Publication No. 2002/0119498, which is incorporated by reference in the present application. Claim 9 has been amended to depend from claim 1 rather than previously cancelled claim 8. No new matter has been added by the amendments and new claims.

Amendment and cancellation of the claims herein are not to be construed as acquiescence to any rejections/objections set forth in the pending Office Action and/or any previous Office Actions and were done solely to expedite prosecution of the application. Applicants reserve the right to pursue the claims as originally filed or similar claims in this or one or more subsequent patent applications.

Applicants respectfully request reconsideration and withdrawal of the objections to and the rejections of this application in view of the amendments and remarks herewith and submit the application is in condition for allowance.

II. The Rejections under 35 U.S.C. § 103 Are Overcome

Claims 1-7, 9, 10, 12-14, 16-19, 21, 22, 26-29, 32-35, and 37 are rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Isalan et al. (Nat. Biotechnol., 2001, 19:656-660; "Isalan") in view of Choo et al. (WO 00/27878; "Choo"). Applicants respectfully disagree and traverse the rejection.

In order to make out a *prima facie* case of obviousness, the Office must show that the claimed invention as a whole, including all elements, would have been obvious to one of ordinary skill in view of the prior art. A clear articulation of the rationale to support a conclusion of obviousness must be supplied, including an assertion of predictability or reasonable expectation of success. *See* MPEP § 2142. In the present case, the references cited by the Office fail to teach or suggest all of the limitations of the claims; there is no sufficient motivation to combine the references; and there is no predictability or reasonable expectation of success.

In view of the Office's interpretation of the claim terms "low-stringency conditions" and "high-stringency conditions", with which applicants do not concur, claim 1 has been amended to recite that the high-stringency conditions are more stringent than the low-stringency conditions. Thus, the invention as currently claimed includes a first round of low-stringency selection using extensively randomized libraries in which a single variable finger has been randomized, followed by a second round of selection under conditions of higher stringency.

As detailed below, none of the cited references, alone or in any combination, teaches or suggests that two rounds of selection under different stringency conditions should be carried out. As the Office action states (at page 9):

> Isalan et al do not explicitly teach using "low stringency conditions" and using a low affinity anchor finger binding sequence (nucleic acid constructs) in the first rounds of selection and later using "high stringency conditions".

The Office action (at page 10) asserts that Isalan "inherently teaches using low or high stringency conditions or using low affinity sequences." However, this assertion is apparently based on the Office's interpretation of the claims prior to the present

amendment. Isalan neither explicitly nor inherently teaches two screening steps, wherein the second step is performed at a higher stringency than the first.

In the alternative, the Office alleges that Choo remedies the deficiencies of Isalan. Choo discloses:

> A method for producing a zinc finger nucleic acid binding protein comprising preparing a zinc finger protein according [to] design rules, varying the protein at one or more positions, and selecting variants which bind to a target nucleic acid sequence by polysome display.

See Choo, abstract. Choo discloses "affinity sharpening" by mutagenesis and subsequent rounds of affinity selection with varied display valency in different rounds of screening (page 27). Choo does not teach or suggest a method with a first selection at low stringency of a set of primary libraries, recombining the nucleic acid sequences from the primary libraries to produce a secondary library encoding multi-zinc-finger polypeptides having zinc-fingers partially optimized for binding to subsites of the sequence of interest, and a second selection at high stringency. Further, Choo only discloses varying the display valency of polysomes (i.e., the number of zinc finger polypeptides displayed per polysome) or the valency of the target nucleic acids. Choo does not teach or suggest varying the buffer conditions, as stated by the Office action at page 10. At page 25, Choo discloses that a non-ionic detergent can be added to the binding and/or wash buffers, but there is no teaching or suggestion that the detergent can be used to alter stringency. At page 27, Choo discloses that ionic strength can be changed to release the RNA component of ribosomes, not for use in selections for zinc finger DNA-binding activities. Therefore, Choo does not make up for the deficiencies of Isalan.

Further, the claimed methods provide advantages not taught or suggested by Isalan or Choo. Most conventional library selection techniques are limited by the need to passage library DNA through bacteria. As one of skill in the art would appreciate, the transformation efficiency of bacteria is currently at best about 10^9-10^{10} transformants per aliquot of DNA per transformation event (*see*, Dani et al., J. Recept. Signal Transduct. Res., 2001, 21:469-488, pages 478-479; submitted herewith). Thus, the effective size of libraries that can be created is about 10^9 at best, given the need to oversample sequence space in order to ensure adequate interrogation of a library.

Isalan and Choo attempt to circumvent this limitation in different ways. Isalan discloses randomizing one-and-a-half fingers out of three in its libraries, a situation requiring randomization of 8 to 10 total residue positions. To limit the size of the libraries, as required by the transformation restriction described above, Isalan imposes a severe limitation on the number of amino acids that can be used at each randomized position (*see*, e.g., Figure 2b). Because of this limitation, the initial libraries of Isalan exclude many potential zinc-finger variants. Choo discloses creating zinc finger polypeptides according to defined rules (*see*, e.g., page 4), and then concentrating mutagenesis at residues –1, +3, +6, and + + 2 (*see*, e.g., page 10). Again, this imposes a severe limitation on the set of zinc fingers that can be produced as only certain positions are randomized, and some of them only in a limited.

The presently claimed methods allow for better querying of the universe of potential zinc finger sequences while accounting for important positional effects between fingers by creating unbiased primary libraries having at least one anchor finger, selecting at low stringency, recombining the sequences that formed binding complexes, and selecting again at higher stringency to produce zinc finger polypeptides that bind with high affinity and specificity to the sequence of interest. By utilizing low stringency for each of the primary selections, each selection yields a pool of zinc finger proteins with target binding affinities that range from low to high. Because of this, there should be no bias towards zinc fingers that bind tightly to their target subsite at the primary selection stage, because zinc fingers so identified may not bind tightly to their target subsite in the context of the zinc fingers selected against the other subsites that make up the full sequence of interest. Additionally, by performing low stringency selections at the primary selections, one can better account for context-dependent interactions between adjacent fingers and can balance the overall affinity and specificity of the fingers in the final array. This allows for more possibilities in the secondary combinatorial library from which to identify combinations that ultimately work well together and can better account for context-dependent effects. None of the cited references, alone or in combination, teaches or suggests such a method that can address all of these issues.

Accordingly, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-7, 9, 10, 12-14, 16-19, 21, 22, 26-29, 32-35, and 37 under 35 U.S.C. § 103(a) over Isalan in view of Choo.

Claims 1-7, 9, 10, 12-14, 16-19, 21-29, 32-35, and 37 are rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Isalan in view of Choo and Isalan et al. (Biochemistry, 1998, 37:12026-33; "Isalan II"). Applicants respectfully disagree and traverse the rejection.

As discussed above, no *prima facie* case of obviousness has been made with regard to claims 1-7, 9, 10, 12-14, 16-19, 21, 22, 26-29, 32-35, and 37 based on the combination of Isalan and Choo. Isalan II does not remedy the deficiencies of these references. Isalan II is cited by the Office (at page 13) as allegedly disclosing "generating codons for all 20 amino acid residues." Isalan II does not teach or suggest the use of more than one selection step or selection at different stringencies.

Further, the combination of Isalan, Choo, and Isalan II would not render obvious claims 23-25. Even, assuming solely for the sake of argument, that it were true that one could theoretically modify the library of Isalan to include between 16 to 20 amino acids at each randomized position, the library would be too large to efficiently query. Using the smaller of the two libraries with 8 randomized positions and assuming only 16 possible amino acids per position, the total number of species in the library would be 16^8, or 4.3×10^9. Using the largest library with 10 randomized positions and assuming 16 possible amino acids per position gives a total number of species of 20^{10}, or 1.0×10^{13}. The combination of Isalan, Choo, and Isalan II does not teach or suggest the claimed methods. Even taken together, the references do not enable or provide any strategy for selecting zinc finger polypeptides from libraries of those sizes.

Accordingly, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-7, 9, 10, 12-14, 16-19, 21-29, 32-35, and 37 under 35 U.S.C. § 103(a) over Isalan in view of Choo and Isalan II.

Claims 1-7, 9, 10, 12-14, 16-19, 21-35, and 37-39 are rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Isalan, Isalan II, and Choo, as applied to claim 1-7, 9, 10, 12-14, 16-19, 21-25, 26-29, 32-35, and 37, and further in view of Joung et al. (Proc. Natl. Acad. Sci. USA, 2000, 97:7382-87; "Joung"). Applicants respectfully disagree and traverse the rejection.

As discussed above, no *prima facie* case of obviousness has been made in view of Isalan, Isalan II, and Choo. Joung does not remedy the deficiencies of these references. Joung discloses bacterial two and one-hybrid systems for use in analysis of polypeptide libraries by selection. Joung does not teach or suggest the use of two screening steps, wherein the second step is performed at a higher stringency than the first, or methods of increasing the effective limits on library size due to cell transformation efficiency. Accordingly, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-7, 9, 10, 12-14, 16-19, 21-35, and 37-39 under 35 U.S.C. § 103(a) over Isalan, Isalan II, and Choo, and further in view of Joung.

Claims 1-7, 9, 10, 12-35, and 37-39 are rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Isalan, Isalan II, Choo, and Joung, as applied to claims 1-7, 9, 10, 11-14, 16-19, 21-35, and 37-39, and further in view of Chandrasegaran (US 6,265,196). Applicants respectfully disagree and traverse the rejection.

As discussed above, no *prima facie* case of obviousness has been made in view of Isalan, Choo, Isalan II, and Joung. Chandrasegaran does not remedy the deficiencies of these references. Chandrasegaran is cited as allegedly disclosing only a specific zinc finger sequence recited in claims 15 and 20. Chandrasegaran does not teach or suggest the use of two screening steps, wherein the second step is performed at a higher stringency than the first, or methods of increasing the effective limits on library size due to cell transformation efficiency. Accordingly, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-7, 9, 10, 12-35, and 37-39 under 35 U.S.C. § 103(a) over Isalan, Choo, Isalan II, and Joung, and further in view of Chandrasegaran.

CONCLUSION

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of all objections and rejections. Applicants respectfully submit that the application is in condition for allowance and request issuance of a Notice of Allowance of the application with all pending claims.

Applicants do not concede any positions of the Office that are not expressed above, nor do applicants concede that there are not other good reasons for patentability of the presented claims or other claims.

If the Examiner is not inclined to allow the application upon consideration of this paper, Applicants respectfully invite the Examiner to contact the undersigned so that a telephonic interview with Examiner can be scheduled prior to issuance of the next Office Action.

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Respectfully submitted,

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